

ABSTRACT

Disclosed are *Hansenula polymorpha* mutants useful as host cells through which various proteins can be produced as being intact at high yield and a process for preparing recombinant proteins using the host cells. Using various vectors, *Hansenula polymorpha* is made to be a mutant which is deprived of methanol assimilating ability and incapable of utilizing methanol as a carbon source. This *Hansenula polymorpha* mutant is used as a high yield host to produce recombinant proteins without continuous feeding of methanol, with the aid of an expression cassette carrying a promoter capable of inducing the expression at a low concentration of methanol. Further, the mutant is also lacking in carboxypeptidase Y, protease Y and/or carboxypeptidase α activity, so the recombinant protein of interest is not degraded at its carboxyl terminal when being expressed in the cell. Thus, intact recombinant protein can be obtained. Also, there is disclosed a pop-out technique in which a recombinant protein expression cassette is inserted into a MOX gene site of the mutant and is allowed to pop out therefrom, thereby utilizing the mutant as a host for general use in producing various proteins of interest.

